

A CONTRIBUTION TO THE FLORAL BIOLOGY AND REPRODUCTIVE SYSTEM OF *COUROUPITA GUIANENSIS* AUBL. (LECYTHIDACEAE)¹

WILMA T. ORMOND, MARIA CÉLIA B. PINHEIRO AND
ALICIA R. CORTELLA DE CASTELLS²

ABSTRACT

Studies of the reproductive system and pollination syndrome of cultivated trees of *Couroupita guianensis* Aubl. are presented. The first report of the internal morphology of the stigma is included. In the stigmatic area two parts can be distinguished, one is hydrophobic and is composed of conspicuous collector hairs and the other is hydrophilic and is formed by the top of the transmission tissue; both parts have important functions in fertilization. The flowers are odoriferous, nectarless and are visited by bees for their pollen. Osmophores are more evident in the top of the filaments of the hood anthers. The pollen is morphologically and physiologically dimorphic. The fertility of the pollen was tested, "in vivo" and "in vitro"; under both conditions, only the staminal ring pollen germinated. The species is self-compatible. Pollen tubes begin to develop in 45 minutes and arrive at the ovules in 24 hours. The field observations and tests demonstrate that the species studied, although allogamous, is self-compatible.

The Neotropical Lecythidaceae have been studied mainly with reference to the floral biology, focusing on the androecium.

Monteiro-Scanavacca (1975), basing her studies on the vascular system of Lecythidaceae species, concluded that in this family the application of the term androphore is erroneous, and defined the structure in question as a body of fused stamens.

Prance (1976) studied the pollination of six Amazonian species of Lecythidaceae from five genera, describing the complex androphore structure and the pollinators. Prance & Mori (1977) discussed androecium and fruit terminology of Lecythidaceae and commented on the similarity between the genera *Lecythis* and *Couroupita*. Mori et al. (1978) classified the New World Lecythidaceae in three basic types according to floral structures: those with actinomorphic androecium being the most primitive (e.g., *Gustavia*), those with zygomorphic hood, flat (e.g., *Couroupita*) and coiled (e.g., *Escheweilera*), being more advanced. Mori & Orchard (1979) described the dimorphic morphology and physiology of the pollen of *Lecythis pisonis* Camb. and compared this pollen with that of *Couroupita guianensis*.

The gynoecium, until now, has only been studied with reference to placentation. Monteiro-Scanavacca (1974), in her studies of 12 species of Lecythidaceae, concluded that the basal portion of the ovary is multilocular with axillary placentation, becoming unilocular with parietal placentation in the distal portion.

In this paper the floral biology and reproductive system are discussed and the stigmatic region, style, and the tissues involved in fertilization are defined. The

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² Museu Nacional—UFRJ. Quinta da Boa Vista, S. Cristóvão, Rio de Janeiro, Brasil.

pollination syndrome and the intrafloral behavior of pollinators are analyzed. We tested the species for autogamy, self-compatibility, and outbreeding. The strong fragrance released by the flower of this species induced us to also study the internal morphology of the osmophores.

MATERIALS AND METHODS

The study material was obtained from 13 trees cultivated in the "Horto Botânico do Museu Nacional" of Rio de Janeiro (Brazil). To study the reproductive system the following tests were used:

Direct Autogamy (Font Quer, 1977).—120 flower buds were enclosed in pollination bags to check for the occurrence of autogamy; 20 of these were observed microscopically to verify pollen deposition on the stigmatic area, while the rest were left bagged.

Self-compatibility.—120 flower buds were enclosed in pollination bags and self-pollination was performed artificially (indirect autogamy); 20 of these flowers were pollinated with pollen from the hood and the remainder, with pollen from the ring. Pollen tube formation and development was observed in fresh sections of the stigma, style and ovary, at different times up to 24 hours after anthesis, for both ring and hood pollen.

Outbreeding.—50 flower buds were bagged and cross-pollinated. Pollen from the ring and the hood was tested with aceto carmine to show presence of significant amounts of protoplasm (Linsley & Cazier, 1963). Pollen tube development was studied in the same manner as described above. Fruit development was observed. The stigmatic area was studied in longitudinal and transverse fresh sections stained with Cotton Blue. The presence of lipids and sugars in stigmatic secretions was detected with Sudan III and Sudan IV and Fehling's reagent (Jensen, 1962). Fertility of hood and ring pollen was determined "in vitro" using the technique outlined by Mori & Orchard (1979). Osmophores were detected with Neutral Red (Vogel, 1963) and thorough studies were made of the internal morphology of different verticils of the floral bud, before and after anthesis.

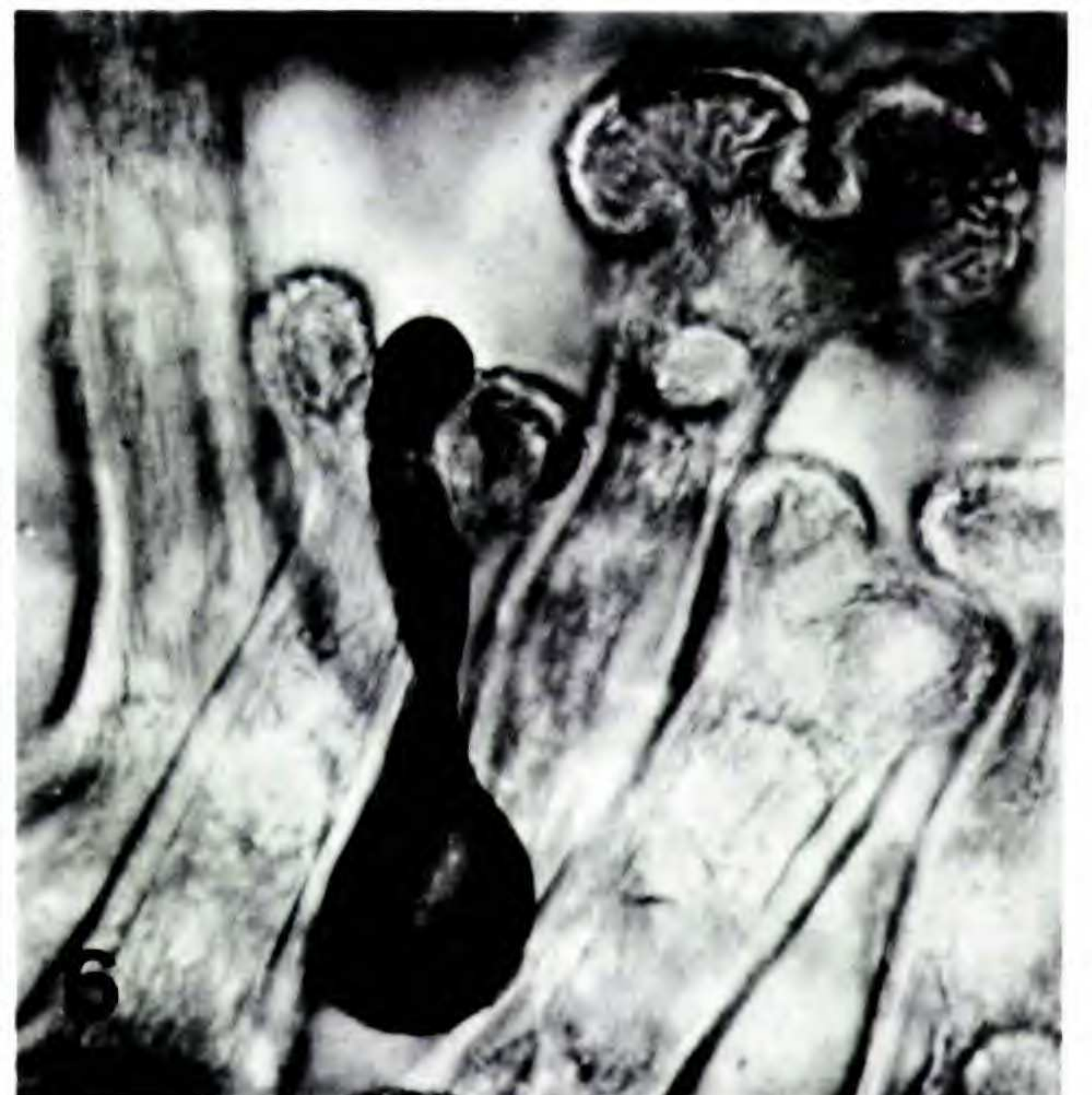
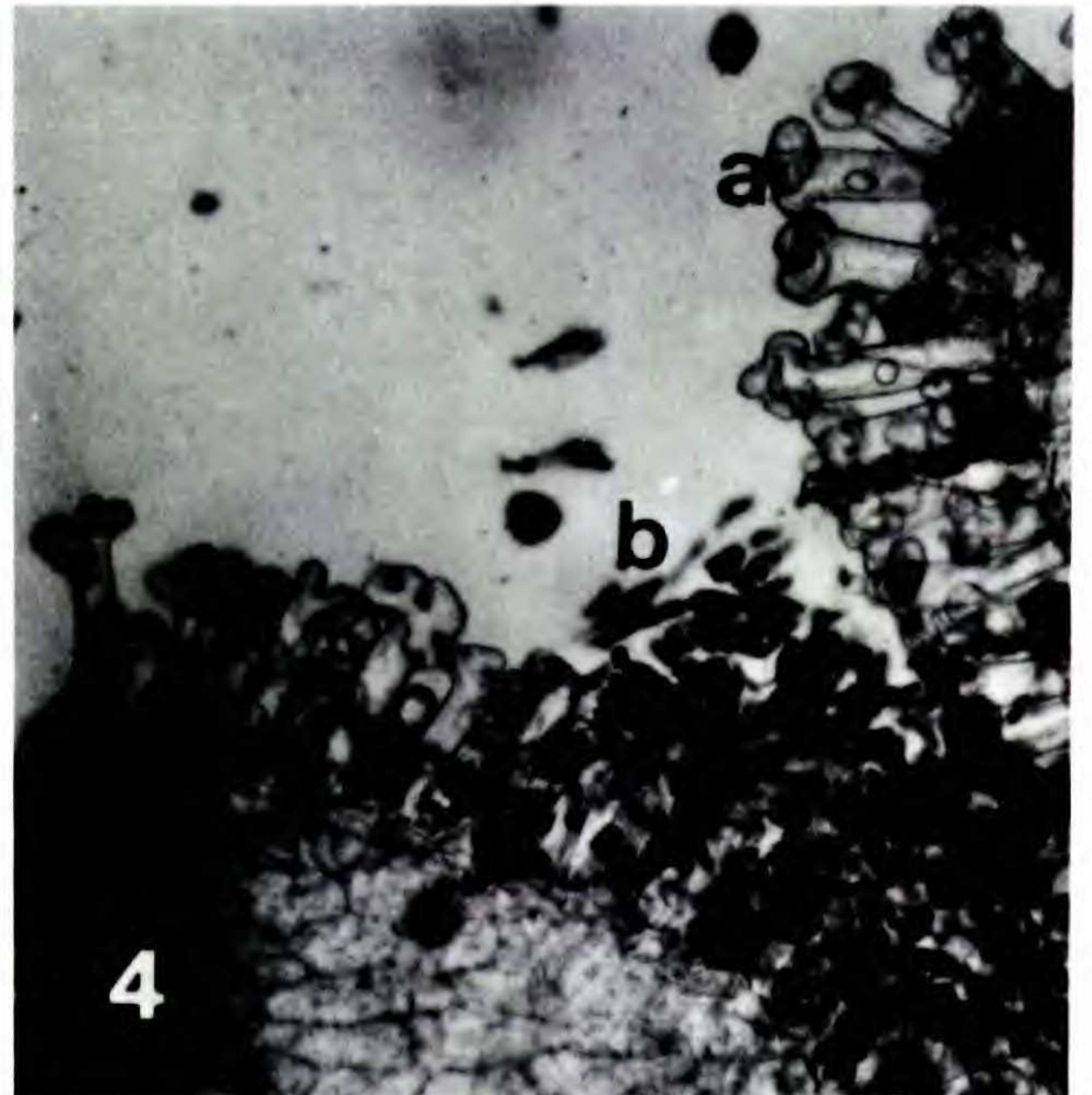
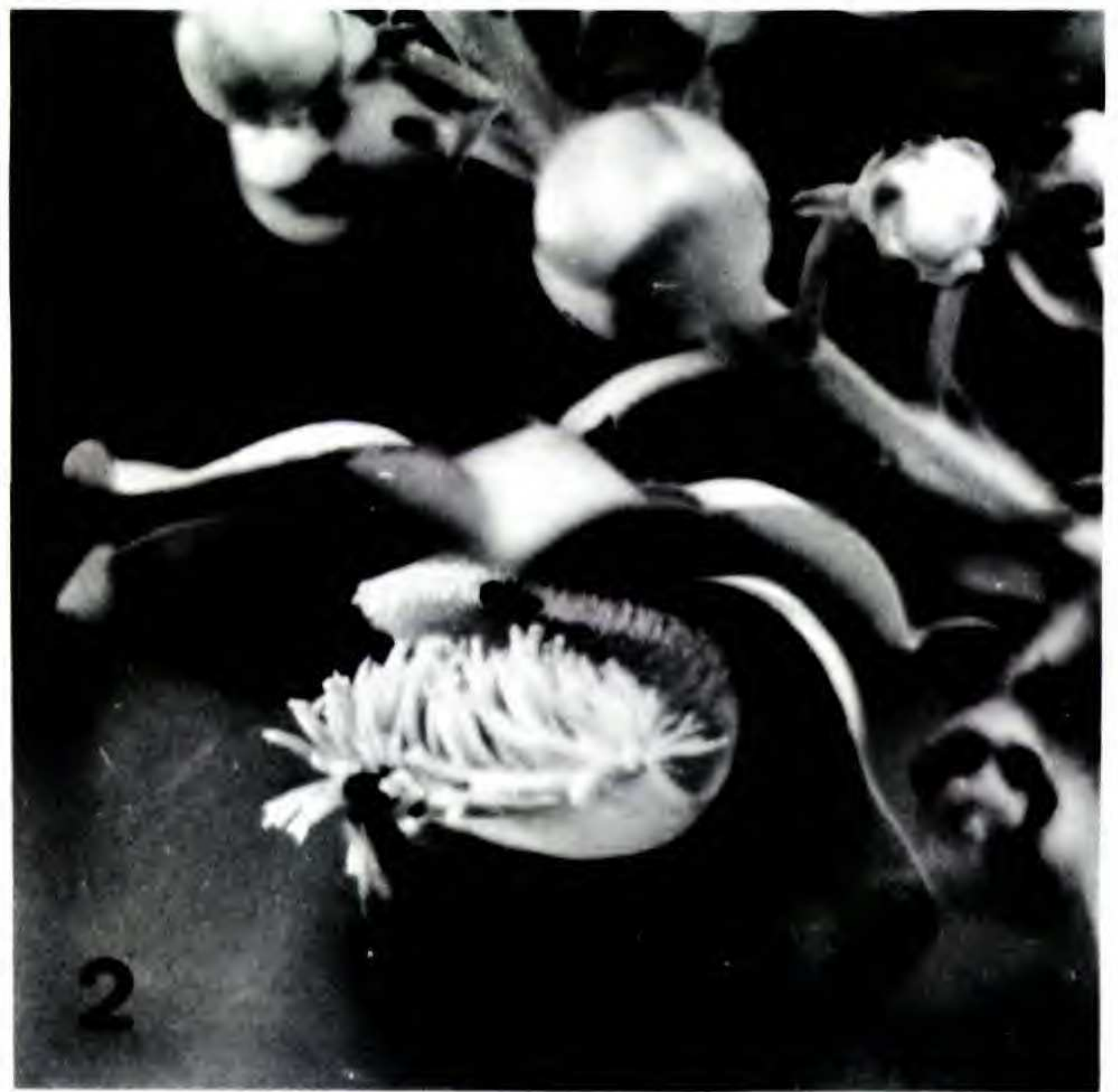
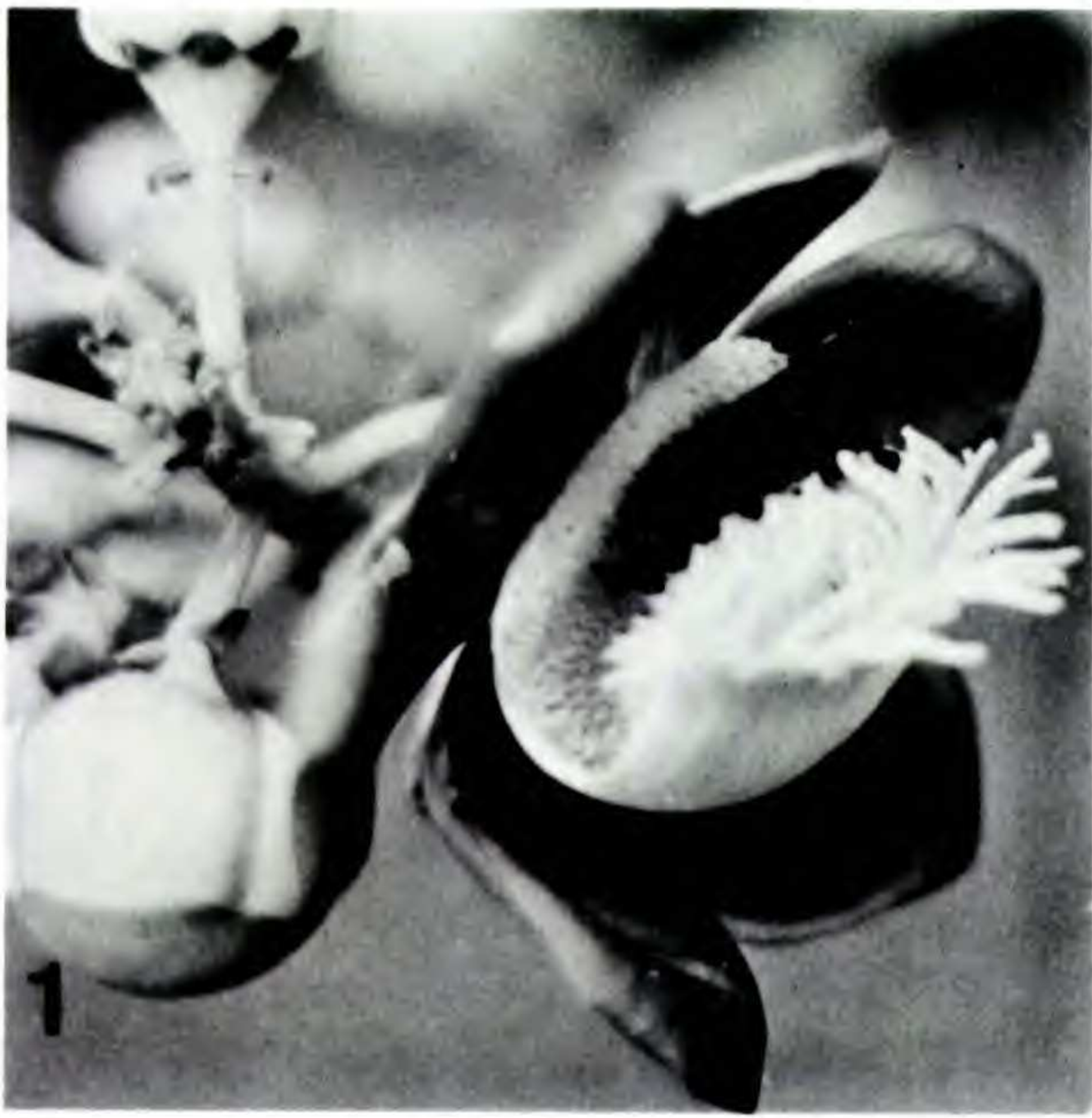
RESULTS, DISCUSSION AND CONCLUSION

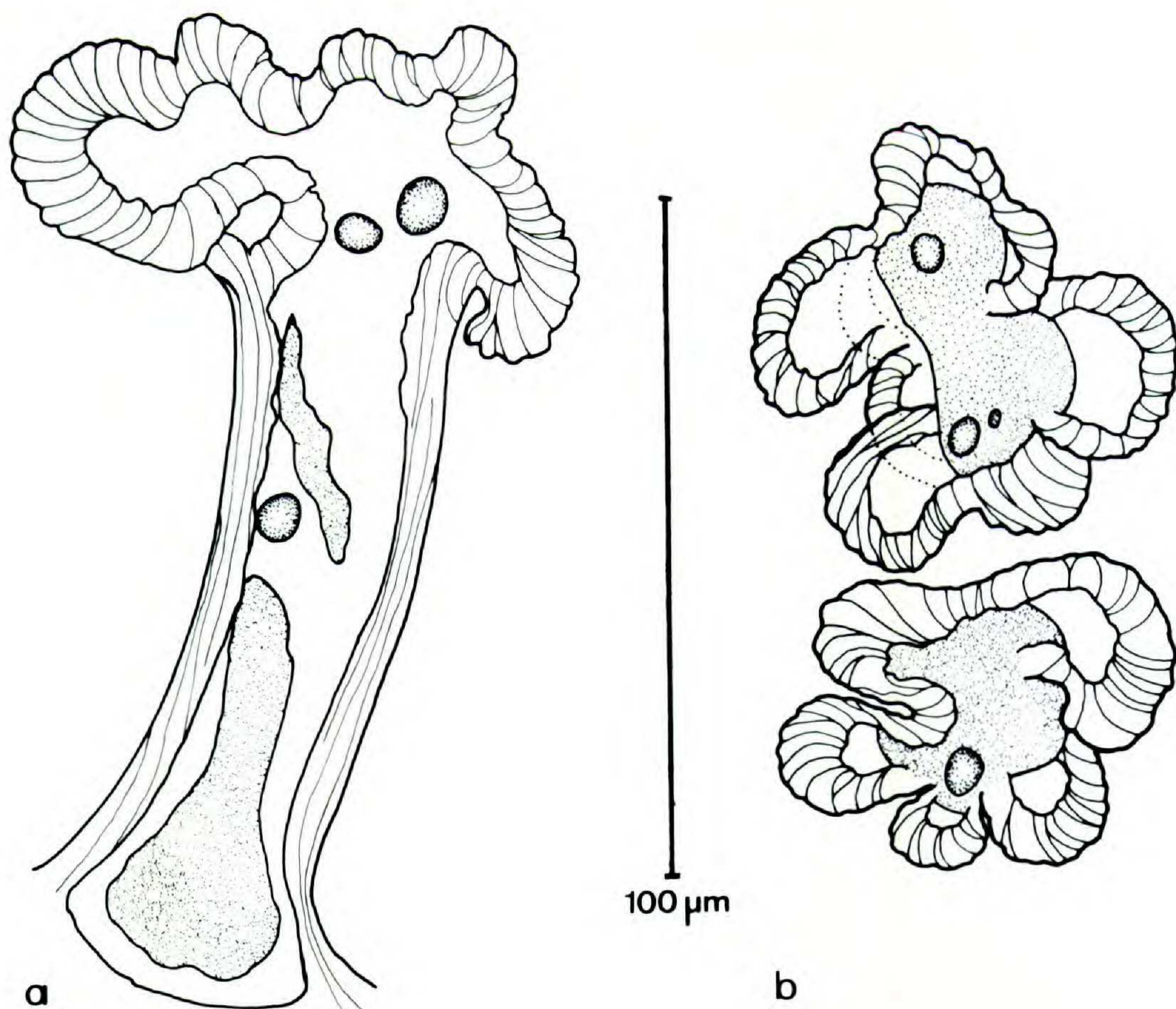
FIELD OBSERVATIONS

The flower of *Couroupita guianensis* is large and strikingly showy with a zygomorphic androecium and a great number of stamens. The corolla has 6–8 petals, the adaxial petal face is purple and the abaxial face is yellow.

Floral patterns, in both visible and ultraviolet light, are very distinctive. Under UV light only the anthers of the hood reflect, presumably serving as pollinator guides. Distinctive floral patterns can serve as guides for a specific pollinator and can be regarded as a potentially important factor in maintaining the separation of sympatric species (Scogin et al., 1977; Jones & Buchmann, 1977). We consider floral patterns as an additional factor to be added to Mori et al.'s (1978) selection of characters that reduce competition for pollinators between different species of Lecythidaceae.

The flowers are odoriferous, nectarless, and are visited by bees for their pollen. Pollen from anthers of the hood constitute a rich source of nutrition for the





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FIGURE 7.—a. Collector hair in lateral view;—b. Collector hair in apical view.

pollinator. We agree with Mori et al. (1978) that the hood plays a dynamic role in pollination strategies. This structure, besides being the most evident in the floral pattern under both visible and UV light, possesses the greatest concentration of osmophores, which are most conspicuous in the filaments. Pollen from the hood anthers is liberated in tetrads and does not germinate. We classify these as “food” anthers similar to those in *Cassia* and *Melastomataceae* (Faegri & van der Pijl, 1971).

Flowers are fully open by 3 A.M. However, the first visits of insects begin around 5 A.M., coinciding with the dehiscence of the anthers. The androecial structure creates a microclimate inside the flower. This is characterized by a saturated humidity in the “cup” formed by the androecium of the ring and hood; condensed droplets are easily observed. This saturated microclimate inhibits anther dehiscence and explains the temporal delay between flower opening and dehiscence.

The flower releases a sweet aroma that is strongest in the early morning. The

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FIGURES 1–6.—1. The effective pollinator: *Xilocopa brasilianorum* L. visiting *Couroupita guianensis*; $\times 0.50$.—2. Bees collecting pollen; $\times 0.50$.—3. Starlike stigma; $\times 33$.—4. Longitudinal section of stigma; $\times 100$:—a. collector hairs;—b. papillose cells of the stigmatic tissue.—5. Collector hairs and individual pollen grains; $\times 400$.—6. Individual pollen germinating; $\times 400$.

presence of strong sweet odor in some genera of Lecythidaceae, including *Couroupita*, has been cited by Mori et al. (1978). In order to locate the structures responsible for this odor in *C. guianensis* the Neutral Red method of Vogel (1963) was used. Because the results of this process proved negative, it was necessary to study the internal morphology of the flower in an effort to find the structures responsible for the odor emission. Osmophores are present in the corolla and in the top of the filaments of the hood anthers, in this place they are more conspicuous (Fig. 12–13). The petals and androecium drop off the plant around noontime.

Many classes of insects may visit the flower (bees, wasps, flies and thrips). *Xylocopa brasiliatorum* L. is the effective pollinator of *C. guianensis* in the area studied. *Apis mellifera* L. may occasionally act as pollinator, especially when numerous individuals visit the flower simultaneously.

Insect visits begin before sunrise and last until noon; however, it is extremely rare to see *X. brasiliatorum* after 10 A.M., behavior that distinguishes it from other bees. The entrance of this bee into the flower is almost always over the frontal part of the hood. This structure functions as a landing platform for *X. brasiliatorum*, while many smaller bees land on the ring or on the hood (Figs. 1–2). In order to enter the flower, *X. brasiliatorum* pushes the hood down causing a release of pollen tetrads that become attached to the ventral part of the insect. The bee only collects pollen from the hood; during the visit its dorsal part rubs against the anthers of the ring. Under pressure, these latter anthers rotate, facilitating the transfer of pollen from both pollen sacs to the insect body. Pollination occurs when the pollen on the dorsal part of the insects touches the stigmatic area that protrudes above the stamens. This type of pollination is nototrobic. The length of the *X. brasiliatorum* visit varies from 30 seconds to 2 minutes.

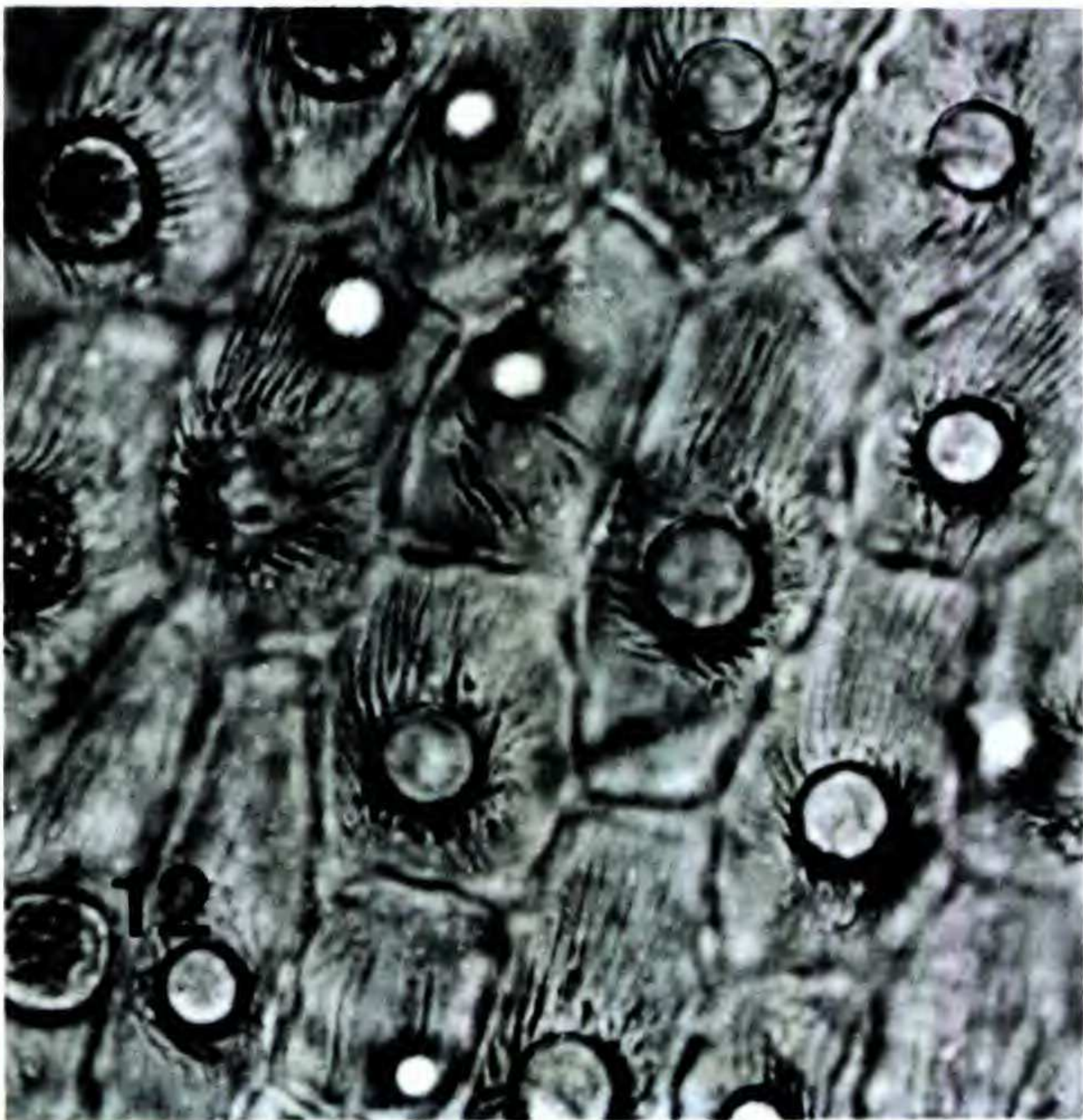
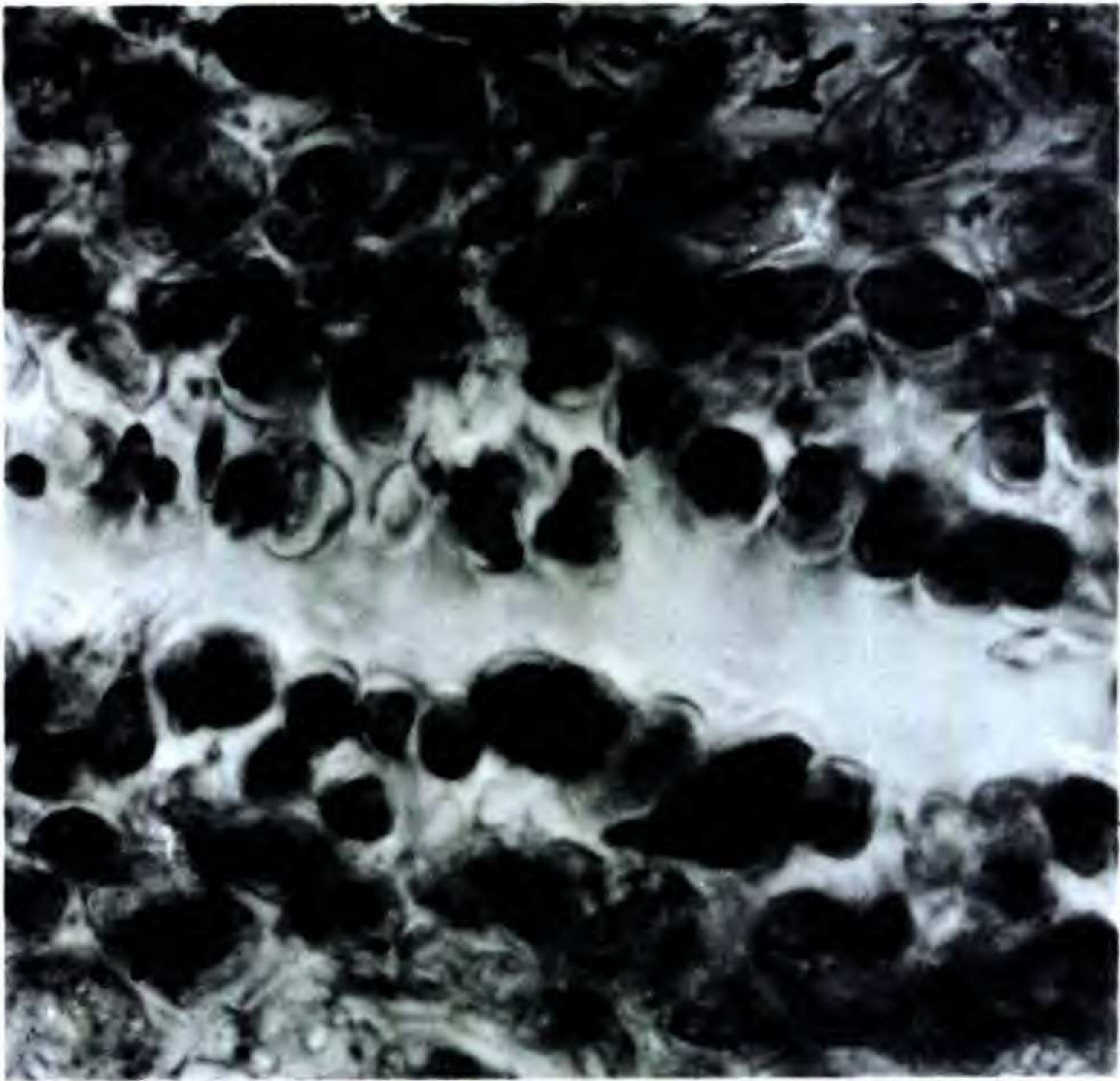
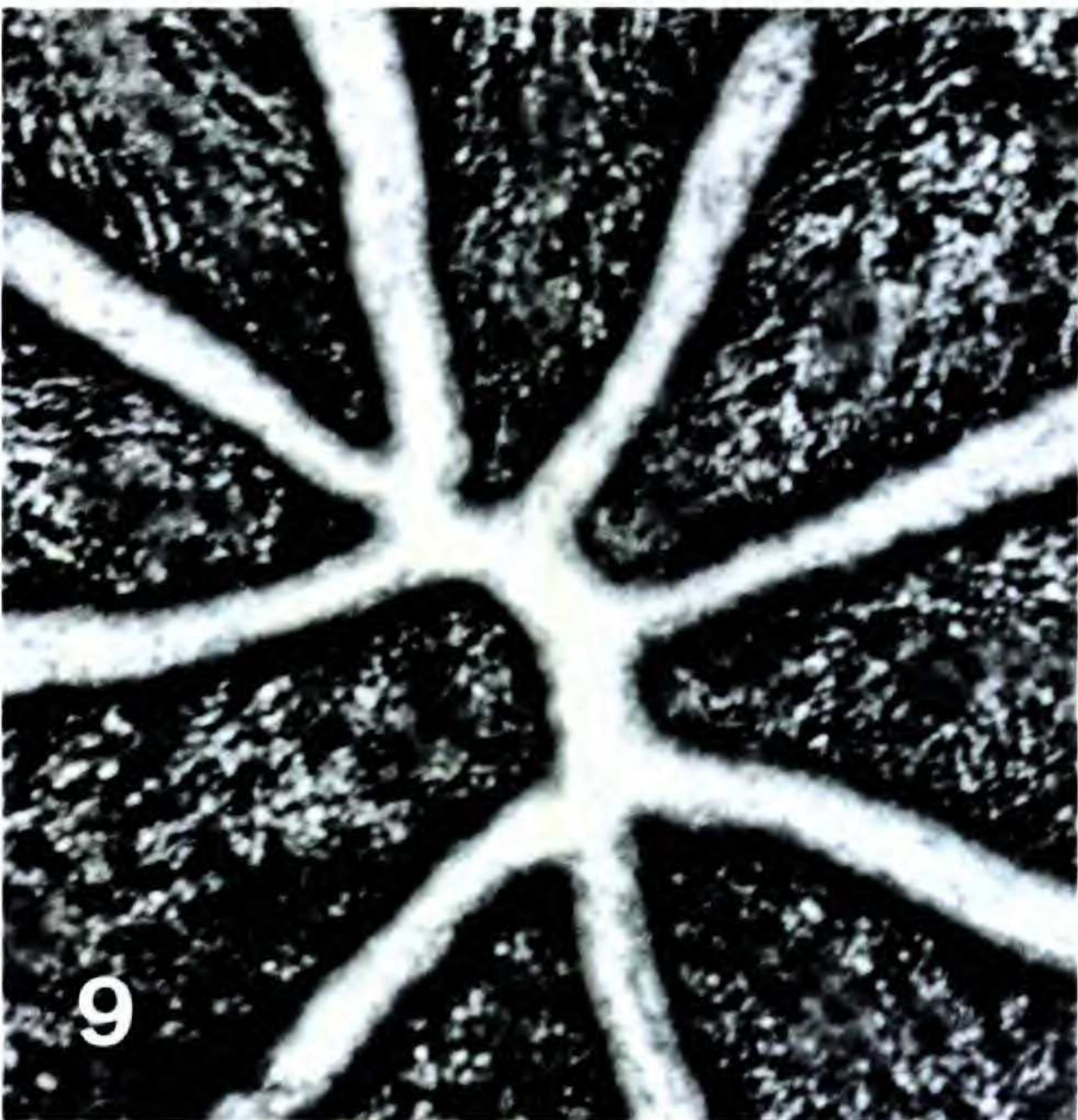
Apis mellifera behaves in a different manner: it collects pollen during early morning hours from the hood, and later in the day, transfers its attention to the ring and occasionally acts as a pollinating vector. Although many others bees of smaller size visit the flower they never touch the stigma and are considered predators.

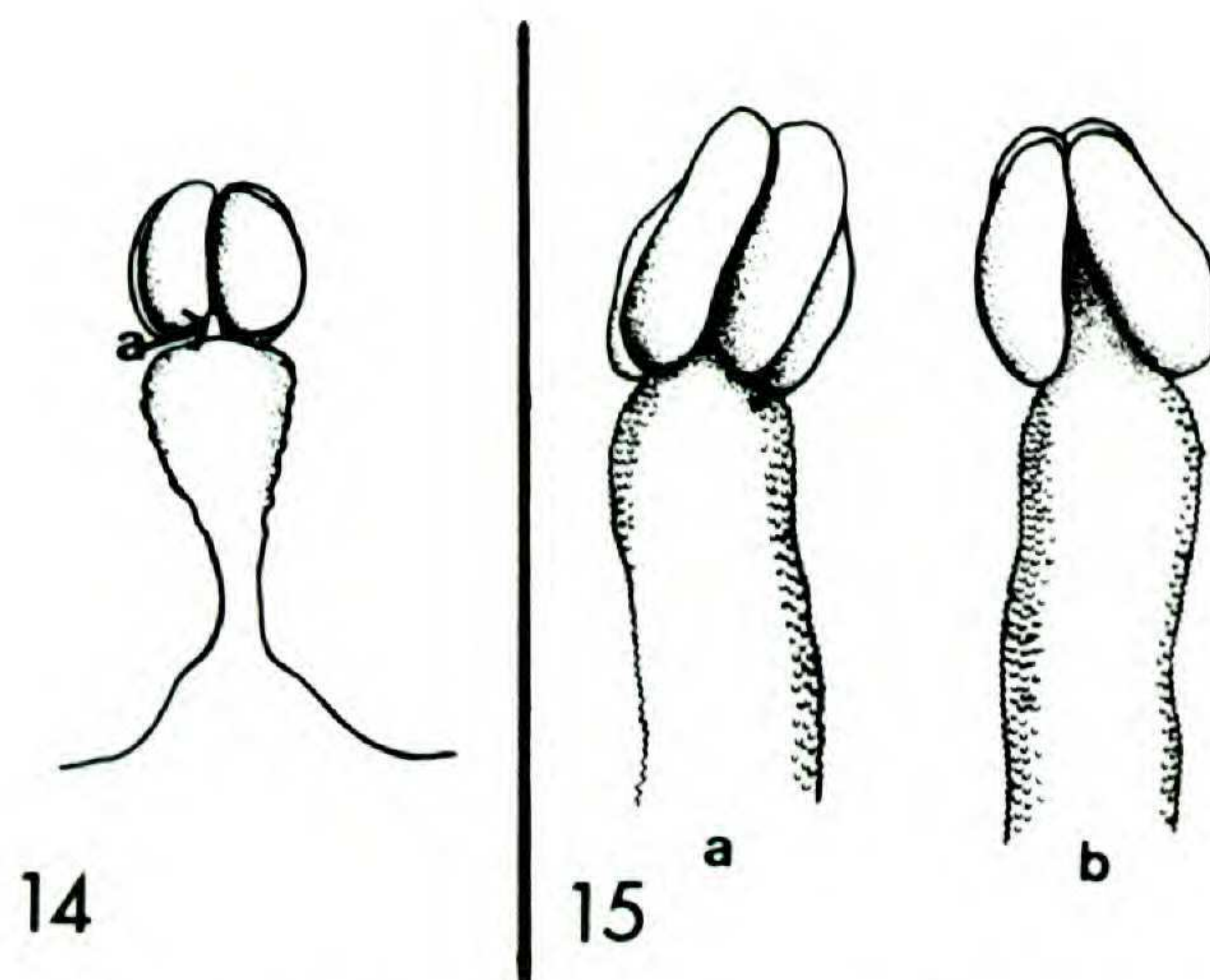
GYNOECIUM

STIGMA.—The stigma exhibits a starlike fissure with six to eight points that correspond to the number of carpels (Fig. 3). Two functionally and structurally different tissues of the stigma, both of which participate in the process of fertilization, can be distinguished (Fig. 4):

Collectors and Secretory Hairs: The hairs are unicellular secreting structures with abundant cytoplasm and thick cellulose walls (Fig. 7a). Distally, they broaden irregularly forming an uneven surface with points and protuberances. The hairs form a dense vesture at the edge of fissures that effectively closes the latter during bud development.

FIGURES 8–13.—8. Top of the transmitting tissue or stigmatic tissue; $\times 400$.—9. Cross section of the style showing canals equal in number equal to the carpels; $\times 40$.—10. Papilionaceous secretory epiderm (transmitting tissue) lining the central canals; $\times 70$.—11. Idem; $\times 400$.—12. The adaxial surface of the corolla—frontal view with oil droplets.—13. Epidermis of the hood filaments; $\times 160$.





FIGURES 14–15. Stamens.—14. Ring stamen;—a. articulations; $\times 12.5$.—15. Hood stamen;—a. frontal view;—b. dorsal view; $\times 12.5$.

The points and protuberances on the superior part of the hairs considerably increase the stigmatic surface. Oil is exuded through pits at the extreme distal end of the hairs (Fig. 7b). Uphof (1962) classified this type of stigmatic hair as pollen collectors. Pinheiro (1979) ascertained the presence of stigmatic hairs with thickened walls in *Cassia ramosa* Vog. var. *maritima* Irwin which, though lacking secretions, functioned mechanically in the collection of pollen.

Examination of longitudinal and transverse sections of the stigma shows the presence on the stigmatic hairs of a great number of adherent isolated pollen grains and only rarely of pollen tetrads. The position of hood anthers in the flower and the behavior of the pollinator prevent pollen of these anthers from reaching the stigma.

The few tetrads that rarely reach the stigma do not adhere because of the topographically irregular distal portion of the stigma, which effectively excludes the tetrads, and because of the absence of a lipid affinity between the hood pollen and the stigmatic hairs (Figs. 5–6).

Papillose Cells (comprising the top of transmitting tissue or stigmatic tissue).—Stigmatic tissue cells are made up of thin-walled papillose cells with abundant cytoplasm and secretions especially of carbohydrates (Fig. 10). It is on this part of the stigma that the pollen germinates. In other plants, the stigmatic tissue can be found totally exposed, forming stigmatic papillae (Arber, 1937; Martin & Ortiz, 1967; Fahn, 1974; Sedgley & Buthose, 1978).

STYLE.—The style has the shape of a truncated cone with the stigmatic surface at the distal end. It is 0.20–0.25 cm long. Cross sections reveal the continuation of stigmatic fissures of constant width through the style. The fissures are lined with a papilionaceous secretory epiderm which provides nutrients for the development of the pollen tube (Fig. 11). This type of syncarpous gynoecium has been described by Fahn (1974). Canals, equal in number to the carpels, traverse the style, leaving a hollow center (Figs. 9–10).

ANDROECIUM

The androecium is characterized by dimorphic pollen and stamens of the ring and hood.

TABLE 1. Experimental results.

Treatment	No. of Flowers	Pollen Tube Development ^a		% Fruit Set	
		SP	TP	Aborted	Mature
Direct self-pollination	100	§	§	0	0
Indirect self-pollination	100	+	—	85	2
Outbreeding	50	+	—	87	6
Natural pollination	100	+	—	88	4

^a SP: single pollen. TP: tetrad pollen. §: stigma without pollen. +: with many pollen tubes. —: without pollen tubes.

In the ring, the anther is attached basifixally to a white filament that is 0.15–0.20 cm long. Its dehiscence is longitudinal and, when totally open, the internal surface of the anther is exposed. Articulation of the anther at the point of contact with the filament allows a swiveling movement that facilitates the transfer of pollen to the dorsal part of pollinator (Fig. 14a). Ring pollen is liberated individually. The exine is smooth as noted by Mori & Orchard (1979) and is covered with droplets of lipid substance that adhere to the insect body. Lipid substances facilitate the clustering of pollen grains (Heslop-Harrison, 1971). The lipophilic character of ring pollen and of stigmatic hairs favor the retention of the former in the stigmatic area. The amount of ring pollen with significant protoplasm was estimated at 95%. Culture of this pollen, “in vitro” and “in vivo,” resulted in the high percentage of germination of about 80%.

The hood filaments are 0.60–1.0 cm long and are purple at the base and yellow at the apex. The white anther is basifixed and dehisces longitudinally (Fig. 15). In contrast with the ring, hood pollen is dry and has a finely sculptured exine. It is released in tetrads and shed in dense “clouds” soon after the flower opens. The amount of hood pollen with significant protoplasm was estimated at 88%. Nevertheless, in “in vivo” and “in vitro” germinating experiments, not one pollen tube was produced. The ring pollen remains in the anthers until about mid-day, while the hood pollen is released within a few hours after the flower opens. In spite of this, the flower continues to be visited by insects other than the pollinators in search of ring pollen.

These results establish evidence for two physiological pollen types, one fertile and the other providing food for the pollinators (Mori & Orchard, 1979).

REPRODUCTIVE SYSTEM

In order to study the reproductive system of *C. guianensis*, individuals were tested for autogamy, self-compatibility and artificial outbreeding. Beyond contributing to the knowledge of the reproductive system of the family, the tests were undertaken to explain the low incidence of fruit production in relation to the great quantity of flowers.

Observations showed that, although the rate of flower fertilization is high, young fruits are aborted in large numbers (Table 1). A parallel study comparing artificially crossed, self-pollinated and naturally pollinated flowers showed that

there is no difference in the rate of pollen germination or of pollen tube growth in the three situations. The test for direct self-pollination (flowers bagged) did not reveal the presence of pollen on the stigma; otherwise in the other tests pollen tubes are well developed in 45 minutes. By following the development of the pollen tube it was seen that after 24 hours all the tubes had reached at least the base of the ovary and some were already present in the micropyle.

Evidently *C. guianensis* does not present pollen antigen-antibody inhibition in the stigma, style, or ovary, as has been described by Lewis & Crowe (1958). The abortion of so many fruits may be because of the tree's incapacity to support additional fruits.

Flowering occurs from the end of November to mid-May. During 1980 a single tree delayed blossoming until the end of May and subsequently set fruit. This fact and that fruit from experimental autogamy grew normally, led us to conclude that *C. guianensis* is self-compatible; this contradicts Mori & Kallunki's (1976) conclusion. Rather the species is allogamic, which coincides with Prance's (1976) observations.

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